

hour. After cooling, the millet grains were separated into six batches and each batch was inoculated with one of the following fungal species:

- A) *Pleurotus ostreatus* (PO, Pearl Oyster)
- B) *Pleurotus pulmonarius* (PP, Phoenix Oyster)
- C) *Pleurotus columbinus* (PC, Blue Oyster)
- D) *Pleurotus eryngii* (PE, King Oyster)
- E) *Ganoderma lucidum* (GL, Reishi)
- F) *Trametes versicolor* (TV, Turkey Tail)

Each batch was then incubated for three weeks until fully colonized by mycelium.

substrate, and sterilization treatment reached about 50% colonization, and again when each jar reached 100% colonization.

### Example 3

**[0059]** Two top-performing fungal species from Example 2 were selected for scale-up testing. Each species will be inoculated onto organic hulled millet that was hydrated to about 60% saturation, sterilized at 250° F. (about 121° C.) for one hour, and cooled to ambient temperature.

**[0060]** Six Substrates were prepared as follows:

Substrate	Substrate					
Component	4	5	6	7	8	9
Sawdust mixture*	0% v/v	10% v/v	0% v/v	10% v/v	25% v/v	50% v/v
Shingle chips**	100% v/v	90% v/v	90% v/v	75% v/v	50% v/v	0% v/v
Shredded wheat straw**	0% v/v	0% v/v	10% v/v	15% v/v	25% v/v	50% v/v

\*Sawdust mixture included 75% alder sawdust, 15% wheat bran, 10% gypsum, and was pre-hydrated to ~65% saturation with water.

\*\*Pre-hydrated by soaking for 1 hour in filtered water.

**[0055]** The following three substrates were prepared:

Substrate Component	Substrate 1	Substrate 2	Substrate 3 (no-asphalt control)
Sawdust mixture*	50% v/v	25% v/v	75% v/v
Shingle chips**	25% v/v	50% v/v	0% v/v
Shredded wheat straw**	25% v/v	25% v/v	25% v/v

\*Sawdust mixture includes 75% alder sawdust, 15% wheat bran, 10% gypsum, and was pre-hydrated to ~65% saturation with water.

\*\*Pre-hydrated by soaking for 1 hour in filtered water.

**[0056]** Eighteen 8-ounce glass jars were filled with about 8 ounces (about 235 mL) of each Substrate. Eighteen jars of each Substrate were sterilized at 250° F. for one hour; eighteen jars of each Substrate were sterilized by steam bath pasteurization; and eighteen jars of each Substrate were not sterilized.

**[0057]** Once all jars reached ambient temperature, each of the six inoculated millet grain batches were added to nine jars at an inoculation rate of about 5% using standard aseptic cultivation techniques as follows:

- 1) Substrate 1, sterilized to 250° F. for one hour via autoclave
- 2) Substrate 1, pasteurized by 160° F. water bath
- 3) Substrate 1, not sterilized
- 4) Substrate 2, sterilized to 250° F. for one hour via autoclave
- 5) Substrate 2, pasteurized by 160° F. water bath
- 6) Substrate 2, not sterilized
- 7) Substrate 3, sterilized to 250° F. for one hour via autoclave
- 8) Substrate 3, pasteurized by 160° F. water bath
- 9) Substrate 3, not sterilized

**[0058]** The inoculated substrate jars were incubated at 70° F. (about 21° C.) for one week. Fungal growth was assessed each day as a function of the degree of colonization observed (see FIG. 2C). Samples were collected for analytical testing at mix-up of the three mixes, when each mix of species,

**[0061]** Twenty-four 5-L containers were filled with each Substrate, for a total of 144 5-L containers. For each Substrate, six containers were sterilized at 250° F. (about 121° C.) for one hour; six were pasteurized using a 160° F. water bath, six were soaked in a strongly alkaline (pH 12) solution, and six were not sterilized.

**[0062]** For each combination of Substrate and sterilization method, three containers were inoculated with each species of incubated grain spawn under aseptic conditions, as follows:

Fungal Species #1—Pearl Oyster (n=72 Containers):

	250° F. @ 1 hour	Pasteurization	Alkaline Soak	No Sterilization
Substrate 4	3 containers	3 containers	3 containers	3 containers
Substrate 5	3 containers (FIG. 3A)	3 containers (FIG. 3D)	3 containers	3 containers (FIG. 3G)
Substrate 6	3 containers (FIG. 3B)	3 containers (FIG. 3E)	3 containers	3 containers (FIG. 3H)
Substrate 7	3 containers (FIG. 3C)	3 containers (FIG. 3F)	3 containers	3 containers (FIG. 3I)
Substrate 8	3 containers	3 containers	3 containers	3 containers
Substrate 9	3 containers	3 containers	3 containers	3 containers

Fungal Species #2—Turkey Tail (n=72 Containers):

	250° F. @ 1 hour	Pasteurization	Alkaline Soak	No Sterilization
Substrate 4	3 containers	3 containers	3 containers	3 containers
Substrate 5	3 containers (FIG. 4A)	3 containers (FIG. 4D)	3 containers	3 containers (FIG. 4G)
Substrate 6	3 containers (FIG. 4B)	3 containers (FIG. 4E)	3 containers	3 containers (FIG. 4H)
Substrate 7	3 containers (FIG. 4C)	3 containers (FIG. 4F)	3 containers	3 containers (FIG. 4I)
Substrate 8	3 containers	3 containers	3 containers	3 containers
Substrate 9	3 containers	3 containers	3 containers	3 containers

**[0063]** After inoculation, each container was incubated at 70° F. (about 21° C.) for three weeks, with fungal growth